

## CRISPR and Agriculture: Transforming Crop Science through Precision Genetics and Sustainable Practices – A Review

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The CRISPR/Cas9 system, short for Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein 9, is a powerful tool for precise DNA editing. It works by targeting specific sequences in the genome using a guide RNA, and the Cas9 enzyme then cuts the DNA at the targeted location. This allows scientists to accurately insert, delete, or modify genetic material. Discovered in 1987 as an adaptive immune system in *Escherichia coli*, the term CRISPR was first coined in 2002 to describe similar structures found in other bacteria and archaea. In 2012, CRISPR was harnessed as a genome-editing tool, and by 2013, its applications in plants were realized. Since then, CRISPR/Cas9 has revolutionized plant genome editing, making the process simpler, more affordable, and more efficient than earlier methods like ZFNs and TALENs, which were both time-consuming and costly. This review provides an overview of the applications of CRISPR/Cas9 in agriculture, highlighting how this genome-editing technique has transformed crop science. It explores the impact of CRISPR/Cas9 on improving crop traits, enhancing pest and disease resistance, increasing environmental stress tolerance, accelerating breeding programs, and boosting yield and quality.

**Keywords:** DNA editing, ZFNs and TALENs, genetic material, crop traits.

### INTRODUCTION

Sustainable agriculture relies on genetic variation in plant crops (Podevin *et al.*, 2013). With the global population projected to reach 9.1 billion by 2050, a 34% increase, the Food and Agriculture Organization of the United Nations (FAO) predicts a 70% rise in the demand for agricultural products. To meet this demand, annual cereal production must increase from 2.1 billion tonnes to approximately 3.0 billion tonnes. As a result, one of the main goals of current agricultural research is to boost productivity, which can be achieved through genome editing techniques, with CRISPR/Cas9 being the most prominent. CRISPR, which stands for Clustered Regularly Interspaced Short Palindromic Repeats, is paired with the Cas9 nuclease, a protein associated with these repeats (Romero *et al.*, 2019). This technology allows for precise genome editing, commonly used in both mammals and plants for research and genetic manipulation. CRISPR/Cas9 is simple, cost-effective, and versatile, making it adaptable to various applications (Song *et al.*, 2016). The CRISPR system was first identified in *Escherichia coli* in 1987 as a type of adaptive immune system that degrades

foreign DNA. The acronym CRISPR was introduced in 2002 after similar structures were found in other bacteria and archaea (Jansen *et al.*, 2002). CRISPR systems are found in about 90% of archaeal and 40% of bacterial genomes (Horvath and Barrangou, 2010). These systems function as a defense mechanism by integrating foreign DNA (spacers) into the CRISPR locus, which then serves as a surveillance system to recognize and degrade foreign nucleic acids in conjunction with Cas proteins. This process occurs in three stages: adaptation (spacer acquisition), expression (production of crRNA), and immunity (interference), where the crRNA and tracrRNA guide the Cas protein to target and cleave foreign DNA (Bhaya *et al.*, 2011).

There are different types of CRISPR/Cas systems, but only the Type II system, which includes Cas9, is widely used for genome editing (Song *et al.*, 2016). Cas9 has a bi-lobed structure, and the guide RNA (sgRNA) is a fusion of crRNA and tracrRNA. This system can edit any sequence in an organism's genome (Romero *et al.*, 2019). Since its introduction in 2012, CRISPR technology has revolutionized life sciences research. CRISPR/Cas9 and CRISPR/Cpf1 are among the most studied and widely used CRISPR systems in

plants (Jinek *et al.*, 2012; Zetsche *et al.*, 2015). Compared to earlier methods like zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), CRISPR/Cas systems have made gene editing more accessible, affordable, and efficient. Although still in its early stages, CRISPR technology is rapidly advancing, with many innovations first reported in animal systems before being applied to plants (Ricroch *et al.*, 2017).

In plants, CRISPR reagents are introduced as DNA, RNA, or protein-RNA complexes, which form an active nuclease at the target site, leading to a double-stranded break (DSB) in the DNA. Plant cells repair these DSBs either through non-homologous end joining (SDN-1 editing), which can result in gene knockouts, or through homologous recombination (SDN-2 and SDN-3 editing), leading to sequence replacements or insertions (Gao, 2018).

CRISPR/Cas9 is a groundbreaking tool for agriculture, allowing scientists to precisely modify crop DNA, enhancing resistance to diseases, pests, and environmental stresses like drought. This technology can also improve the nutritional content and productivity of crops, contributing to food security. CRISPR/Cas9 has been applied to both monocot and dicot crops, improving yield, quality, disease resistance, and climate resilience (Ma and Liu, 2016). The genomes of cereal crops such as wheat, corn, rice, as well as fruits and vegetables like potatoes, tomatoes, bananas, and apples, have been edited using CRISPR/Cas9 (Ricroch *et al.*, 2017; Zhang *et al.*, 2016). One of the most common applications is gene knockdown, achieved by introducing indels that cause frameshift mutations, leading to the development of numerous beneficial traits in various crops (Rasheed *et al.*, 2021).

**Applications of CRISPR/Cas9 technology in tomato (*Solanum lycopersicum*):** In many parts of the world, fruits are staple foods and crucial sources of essential nutrients. With the challenges posed by a growing global population and climate change, it is imperative to develop fruit crops that not only yield high quantities but also exhibit enhanced resilience to environmental stresses. Traditional breeding methods are unlikely to meet this increasing demand (Wang *et al.*, 2018). To address these challenges, ongoing technological innovation is essential (Bigliardi and Galati, 2013). Over the past two decades, genetic engineering techniques have significantly advanced fruit crop production, improving key agronomic traits such as fruit quality and stress tolerance. Recombinant DNA technology, in particular, enables the transfer of beneficial genes from various organisms, plants, or microorganisms, thereby offering opportunities to enhance fruit yield, quality, and shelf life. As a result, genetic engineering has emerged as one of the fastest-developing technologies in agriculture (Parmar *et al.*, 2017).

According to Duane Green, a fruit crop is a perennial edible crop where the economic yield is derived from either the botanical fruit or a byproduct of it (Reiger, 2006). Additionally, certain plants like melons, tomatoes, and cucumbers, typically grown as annuals, are also classified as fruit crops (Miller and Gross, 2011). Among these, the tomato serves as a model organism for studying fruit biology due to its accessible germplasm resources, simple diploid inheritance, efficient breeding, short growth cycle, ease of genetic modification, and extensive research base (Wang *et al.*, 2018). The applications of the CRISPR-Cas9 system in improving tomato fruit crops are discussed in detail, as outlined in Tables 1, 2, and 3.

**Table 1. Application of CRISPR/Cas9 against biotic stress in different crops.**

Crop species	Targeted genes	Targeted traits	References
Tomato	DCL2	Susceptibility to potato virus X, tobacco mosaic virus, and tomato mosaic virus	(Wang <i>et al.</i> , 2018; Wang <i>et al.</i> , 2018)
Tomato	DMR6	Resistance against downy mildew	(Thomazella <i>et al.</i> , 2016)
Tomato	MAPK3	Susceptibility to grey mold disease	(Zhang <i>et al.</i> , 2018)
Tomato	S1PLC2	Resistance against pathogen	(Perk <i>et al.</i> , 2023)
Rice	OsDjA2 and OsERF104	Resistance against blast disease	(Távora <i>et al.</i> , 2022)
Rice	Bsr-d1, Pi21 and ERF922	Resistance against blast disease	(Zhou <i>et al.</i> , 2022)
Rice	OS8N3	Resistance against bacterial blight	(Kim <i>et al.</i> , 2019)
Rice	OsSWEET114	Resistance against bacterial blight	(Zafar <i>et al.</i> , 2020)
Rice	OSPU9	Resistance against bacterial leaf blight	(Kim <i>et al.</i> , 2024)
Maize	LIPOXYGENASE3	Increase tolerance by reducing ROS accumulation	(Pathi <i>et al.</i> , 2020)
Maize	ZMCOI10	Increase maize immunity	(Ma <i>et al.</i> , 2021)
Wheat	TaPDIL1-5	Encodes protein disulfide isomerase like 5-1	(Kan <i>et al.</i> , 2022)
Wheat	TaCIPK14	Encodes a protein that belongs to the CBL interacting protein kinase	(He <i>et al.</i> , 2023)
Wheat	TaNFXL1	Negatively regulates trichothecene induced defense responses	(Brauer <i>et al.</i> , 2020)



**Table 2. Application of CRISPR/Cas9 against abiotic stress in different crops.**

Crops	Targeted gene	Targeted traits	References
Tomato	BZR1	Decrease in heat stress tolerance	(Li <i>et al.</i> , 2018)
Tomato	SILBD40	Increase drought resistance	(Liu <i>et al.</i> , 2020)
Tomato	APX2	Increase tomato thermotolerance	(Hu <i>et al.</i> , 2021)
Tomato	SINPR1	Cold stress tolerance	(Shu <i>et al.</i> , 2023)
Rice	OS6HLHO24	Increase in total chlorophyll content and shoot biomass upon exposure to salt stress	(Alam <i>et al.</i> , 2022)
Rice	OsPRX2	improves potassium deficit tolerance	(Mao <i>et al.</i> , 2019)
Rice	OSALS1	Increase herbicide tolerance	(Kuang <i>et al.</i> , 2020)
Rice	OSPUB7	Increase drought tolerance	(Kim <i>et al.</i> , 2023)
Rice	OSDSG1	Increase salt tolerance	(Ly <i>et al.</i> , 2024)
Maize	ZmALS1	Increase Chlrosulfuron resistance	(Li <i>et al.</i> , 2020)
Maize	ZmHKT1	Increase salt tolerance	(Zhang <i>et al.</i> , 2020)
Maize	ZmTS5	Increase resistance against heat	(Mohamed <i>et al.</i> , 2024)
Maize	MS26	Increase resistance against herbicides	(Mohamed <i>et al.</i> , 2024)
Maize	ARGOS8	Increase drought tolerance	(Mohamed <i>et al.</i> , 2024)
Wheat	TaERF3	Increases salt and drought stress tolerance	(Debbarma <i>et al.</i> , 2019)
Wheat	TaMBR1c	Enhances heat stress tolerance	(Yu <i>et al.</i> , 2022)
Wheat	SAI1	Enhances drought stress tolerance	(Abdallah <i>et al.</i> , 2022)

**Table 3. CRISPR/Cas9 applications in tomato quality and yield improvement.**

Targeted genes	Targeted traits	References
CLV3	Fruits with increasing locule numbers	(Sethi, 2024)
MYB12	Pink-colored tomato	(Yang <i>et al.</i> , 2019)
MPK20	Repression of genes controlling sugar metabolism	(Chen <i>et al.</i> , 2018)
ALMT9	Decrease in malate content	(Wang <i>et al.</i> , 2018)
INVINH1, VPE5	Elevated level of glucose, fructose and TSS	(Wang <i>et al.</i> , 2022)
CS2	Increase in grain weight, size and yield	(Wang <i>et al.</i> , 2022)
OSAAP11	Increase in rice grain quality	(Yang <i>et al.</i> , 2023)
EHD1	Enhance grain quality and yield	(Song <i>et al.</i> , 2024)
ZmACO2	increases in the quantity of kernels, ear weight, and ear length	(Li <i>et al.</i> , 2017; Li <i>et al.</i> , 2020)
ZmWAX1, ZmSH2, ZmbZIP22, ZmBADH20, ZmBADH26	Enhance nutritional value and quality	(Jiang <i>et al.</i> , 2022)
Wx genes	Key enzyme in amylase biosynthesis	(Maryami <i>et al.</i> , 2020; Zhang <i>et al.</i> , 2021)
WxA1, WxB1, WxD1		
TaASN2	Encoded Asparagine synthetase	(Raffan <i>et al.</i> , 2021)
TaIPK1	Increase in iron and zinc accumulation	(Ibrahim <i>et al.</i> , 2022)
Tamyb10	Pre-harvest sprouting resistant	(Zhu <i>et al.</i> , 2023)
TaSPL13	Regulate flowering time and various developmental processes	(Gupta <i>et al.</i> , 2023)
TaARF15-A1	Negatively regulates senescence	(Li <i>et al.</i> , 2023)
TaRPK1	Enhance yield	(Rahim <i>et al.</i> , 2024)

**Resistance to biotic stresses:** Biotic stressors such as insects, fungi, bacteria, and viruses can severely impact plant health. Since its successful application in creating stable transgenic lines in 2013, CRISPR-Cas9 technology has been instrumental in developing disease-resistant plants. This technology has been used to combat bacterial, fungal, and viral infections, which cause significant losses in tomato crops (Wang *et al.*, 2018).

**Two primary approaches have been employed to fight viruses:** The creation of single-stranded RNAs (sgRNAs) that

target the virus genome directly through sequence complementation and the modification of tomato genes to confer antiviral properties. For example, Tashkandi *et al.* (2018) used the CRISPR-Cas9 system to develop tomato plants resistant to the tomato yellow leaf curl virus. This approach resulted in decreased viral genomic DNA accumulation and effective viral interference compared to wild-type plants. Additionally, CRISPR-Cas9 has been used to knock out genes like Tomato Dicer-like 2 (DCL2), which play a role in resistance pathways. DCL2 mutants showed



susceptibility to infections by tobacco mosaic virus, tomato mosaic virus, and potato virus X, indicating DCL2's role in defending against RNA viruses (Wang *et al.*, 2018).

Tomato crops are also vulnerable to significant economic losses due to downy and powdery mildews. The Downy Mildew Resistant 6 (DMR6) gene in *Arabidopsis thaliana* is involved in salicylic acid homeostasis, and its overexpression increases susceptibility to downy mildew. DMR6, part of the 2-oxoglutarate Fe (II)-dependent oxygenase superfamily, has been studied in tomatoes. By deactivating the DMR6 ortholog in tomatoes using CRISPR-Cas9, researchers found that *dmr6* mutants exhibited resistance to various pathogens, such as *Pseudomonas syringae*, *Phytophthora capsica*, and *Xanthomonas* spp., without significant negative effects (Thomazella *et al.*, 2016). Similarly, grey mold caused by *Botrytis cinerea* is a common postharvest infection in tomatoes. Using CRISPR-Cas9 technology, researchers have demonstrated that Mitogen-Activated Protein Kinase 3 (MAPK3) confers resistance to *B. cinerea* (Zhang *et al.*, 2018). Additionally, CRISPR-Cas9 was used to inactivate the tomato Phospholipase C2 gene (SIPLC2), implicated in plant resistance to diseases like *B. cinerea*. The resulting SIPLC2-knockout tomato lines showed reduced ROS generation, leading to increased resistance and lower pathogen growth (Perk *et al.*, 2023).

**Resistance to abiotic stresses:** Charles Darwin's theory of evolution highlights that the most robust species can adapt to their changing environments, ensuring their survival. Abiotic stressors like heat, cold, drought, and floods are significant challenges for crops. While traditional breeding methods have improved crop productivity, new strategies are needed to boost output further. CRISPR-Cas9 technology shows great promise in this regard (Wang *et al.*, 2018).

The Brassinazole Resistant 1 (BZR1) gene is involved in brassinosteroid (BR) signaling and regulates the BR response. Its homolog in tomatoes also affects thermotolerance and modulates the BR response. Cold sensitivity in tomatoes can negatively impact fruit quality (Li *et al.*, 2018). C-repeat Binding Factor 1 (CBF1) plays a protective role against cold injury, while MAPK3 is involved in the tomato drought response by protecting cell membranes from oxidative damage (Zhang *et al.*, 2018). Knocking down SILBD40 (Lateral Organ Boundaries Domain Transcription Factor) in tomatoes increases drought tolerance (Liu *et al.*, 2020). The CRISPR/Cas9 system has also enhanced tomato thermotolerance by targeting the APX2 (Ascorbate Peroxidase 2) gene through CPK28 (Calcium-Dependent Protein Kinase 28) (Hu *et al.*, 2021). Additionally, CRISPR/Cas9-mediated editing of the SINPR1 gene has been linked to chilling injury in tomatoes, as evidenced by reduced proline content, antioxidant enzyme activity, and soluble protein content, along with the accumulation of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), superoxide anion (O<sub>2</sub><sup>-</sup>), and malonic dialdehyde (MDA) (Shu *et al.*, 2023).

**Improvement in tomato quality:** Tomato fruit quality is assessed by external characteristics such as texture, color, and size, as well as internal factors like sugar and vitamin levels and beneficial compounds like malate, anthocyanin, and lycopene (Satpute and Jagdale, 2016).

Tomato yield, a crucial trait in crop breeding, is influenced by factors such as fruit-setting rate, flowering speed, and the number and size of final fruit cells. Genetic studies have identified specific genes associated with yield (Ariizumi *et al.*, 2013). CRISPR/Cas9-induced mutations in genes like CLV3, Compound Inflorescence (S), and Self-Pruning (SP) have been shown to increase the size of fruit or floral organs, leading to higher tomato yields (Sethi, 2024). Various factors contribute to fruit quality, including color, nutrients, size, shape, sweetness, acidity, aroma, and shelf life. While red tomatoes are the norm, there is a growing demand for other colors (Zhang *et al.*, 2023). CRISPR/Cas9 has been used to target the MYB12 gene, modifying flavonoid biosynthesis to produce pink tomatoes, particularly popular in Asia (Yang *et al.*, 2019). Similarly, gene editing in the carotenoid production pathway has yielded orange and yellow tomatoes. Anthocyanin-rich purple tomatoes, like the "Sun Black" variety, have also been developed to cater to consumer preferences (Liu *et al.*, 2021).

Enhancing the nutrient and bioactive content of fruits is another focus of tomato improvement. Vitamins and carbohydrates are vital for energy, and multiple genes are involved in the production and metabolism of sucrose and carotenoids. Mutations in Mitogen-Activated Protein Kinase 20 (MPK20) have altered the expression of these genes (Chen *et al.*, 2018). Through CRISPR-Cas9, tomato fruits have been engineered to boost anthocyanins, GABA, and lycopene levels by manipulating key genes in their metabolic pathways. Additionally, CRISPR-Cas9 has been used to identify ALMT9 (Aluminum-Activated Malate Transporter 9), the main gene affecting tomato malate concentration (Wang *et al.*, 2018). CRISPR-mediated knockout mutants of the INVINH1 and VPE5 genes have led to significantly higher levels of glucose, fructose, and total soluble solid (TSS) content (Wang *et al.*, 2022).

**CRISPR/Cas9 and rice crop enhancement:** Rice is a critical staple food, ensuring food security for nearly half of the world's population. The CRISPR/Cas9 technology offers a powerful tool for crop development by precisely breaking DNA sequences at targeted sites, enabling the enhancement of rice productivity and resilience to both biotic and abiotic stresses. By using rice as a model plant for functional genomic analysis, this technology not only advances rice cultivation but also provides insights applicable to other cereal crops. The increasing specificity of CRISPR/Cas9 in genome editing has significantly facilitated molecular breeding techniques and the identification of gene functions, enabling the rapid creation of improved cultivars with higher yields and other





desirable traits, crucial for sustainable agricultural production (Gurunathan *et al.*, 2023).

**Resistance to biotic and abiotic stresses:** Biotic and abiotic stresses are major challenges that hinder optimal crop output during both vegetative and reproductive stages. CRISPR/Cas9 technology has proven effective in addressing these challenges, as summarized in Tables 1 and 2.

**Applications in biotic stress:** One of the most severe diseases affecting rice is blast, caused by *Pyricularia oryzae* (syn. *Magnaporthe oryzae*), which significantly reduces crop yields globally (Jain *et al.*, 2017). The most sustainable method to combat this disease is the cultivation of rice varieties with multiple resistance genes. For example, shotgun proteomics identified OsDjA2 and OsERF104 as potential targets for enhancing resistance to *P. oryzae* infection. CRISPR/Cas9-mediated deletion of these genes in mutant Nipponbare rice plants resulted in significantly lower disease symptoms compared to control plants (Távora *et al.*, 2022). Additionally, CRISPR/Cas9 was used to create single and triple mutants in an indica TGMS line (LK638S), targeting the genes Bsr-d1, Pi21, and ERF922. Among these, the ERF922 mutant demonstrated the strongest resistance to blast disease (Zhou *et al.*, 2022). In another study, the Os8N3 gene in *Oryza sativa* L. ssp. Japonica was targeted to enhance resistance to bacterial leaf blight, a significant rice disease, by editing the promoter region of OsSWEET14 genes in Basmati varieties using CRISPR/Cas9 (Zafar *et al.*, 2020). Further, the OsPUB9 gene was edited to increase resistance to bacterial leaf blight, with CRISPR/Cas9-OsPUB9 vectors introduced into plants via an Agrobacterium-mediated method (Kim *et al.*, 2024).

**Applications in abiotic stress:** Abiotic stresses, such as drought and salinity, are exacerbated by factors like unbalanced hormone levels, reduced antioxidant effects, and increased ROS production (Abd El Mageed *et al.*, 2023). CRISPR/Cas9 has been instrumental in enhancing rice tolerance to these stresses. For instance, downregulating genes such as OsDIS1 (drought-induced SINA protein 1), OsDST (drought and salt-tolerant protein 1), and OsSRFP1 (ring finger protein 1) has led to increased antioxidant enzyme levels and decreased H<sub>2</sub>O<sub>2</sub> concentrations, thereby enhancing drought resilience (Kumar *et al.*, 2020). Additionally, editing the OsbHLH024 gene resulted in rice mutants with increased chlorophyll content and biomass under salt stress, even with minimal DNA alterations (Alam *et al.*, 2022). CRISPR/Cas9-based editing has also been used to improve potassium deficiency tolerance by targeting OsPRX2, which reduces ROS production and enhances stomatal closure, increasing stress tolerance (Mao *et al.*, 2019). Furthermore, CRISPR/Cas9 has been applied to develop herbicide-resistant crops by editing the OsALS gene, as demonstrated in *Oryza sativa*, where herbicide tolerance was introduced without compromising yield (Kuang *et al.*, 2020). A recent study focused on targeted mutation of the OsPUB7 gene to create

drought-resistant rice (Kim *et al.*, 2023). Moreover, the CRISPR/Cas9-induced mutation in the OsDSG1 gene, involved in ubiquitination and metabolic processes, resulted in enhanced salt tolerance in rice mutants, evidenced by improved plant height, root length, and other growth parameters (Ly *et al.*, 2024).

**Yield improvement:** Grain yield is a complex trait and a primary focus of rice crop enhancement. Polygenes control rice yield potential, with significant contributions from grain number per panicle, panicle number per plant, and 1,000 grain weight (Xing and Zhang, 2010). The CRISPR/Cas9 framework has demonstrated its potential to boost rice grain yield by targeting genes such as Gn1a, DEP1, GS3, and IPA1, which regulate plant and panicle architecture as well as grain number per panicle (Shan *et al.*, 2014). Significant interest exists among molecular biologists and breeders in further enhancing yield through CRISPR/Cas9 (Gurunathan *et al.*, 2023). For instance, targeting the miR396 site in the CS2 gene, which influences seed shattering, grain size, and abiotic stress response, resulted in a GS2E mutant with improved grain size and yield, increasing the 1,000 grain weight by 23.5% and overall yield by 10.4% (Wang *et al.*, 2022). Additionally, rice grain quality was improved by knocking out the OSAAP11 gene using CRISPR/Cas9 technology (Yang *et al.*, 2023). In Japonica rice varieties, editing the EHD1 gene through CRISPR/Cas9 led to enhancements in both yield and grain quality (Song *et al.*, 2024).

**CRISPR/Cas9 and Maize Crop: Revolutionizing corn agriculture through genome editing:** Maize (*Zea mays*) has been a vital crop for thousands of years, originating in the Americas over 9,000 years ago and becoming a cornerstone of agricultural societies worldwide. Today, maize serves multiple purposes, including as food, animal feed, and a raw material for various industrial products. Its significance in agriculture and global food security is immense. As a C4 photosynthetic plant, maize efficiently converts carbon dioxide into organic molecules, contributing to its high yield potential. Its adaptability to diverse climates and growing conditions further underscores its importance. Additionally, maize is crucial for the livestock sector and for producing industrial products, starches, oils, biofuels, and animal feed (Ammar *et al.*, 2023). As the global population grows, the agricultural sector faces the challenge of producing more food with reduced environmental impact and resource use. Therefore, advancing maize agriculture through innovative technologies like CRISPR/Cas9 has become increasingly relevant (Lu *et al.*, 2023). This technology is widely applied in maize breeding, with applications detailed in Tables 1, 2, and 3.

**Resistance to biotic stress:** CRISPR/Cas9 has been pivotal in developing maize with enhanced resistance to biotic stressors such as fungal pathogens and pests. The smut fungus *Ustilago maydis* and the fusarium fungus *Fusarium graminearum* are major threats that significantly reduce maize yield and quality



(Wu *et al.*, 2018; Pathi *et al.*, 2020). For instance, the knockout of the LOX3 gene, which is upregulated during *U. maydis* infection, has shown reduced susceptibility in maize mutants due to decreased reactive oxygen species (ROS) accumulation (Pathi *et al.*, 2020). Additionally, CRISPR/Cas9 has been used to modify genes involved in the jasmonate signaling pathway, such as ZmCOI1a and ZmJAZ15. The ZmCOI1a mutant demonstrated increased resistance to *F. graminearum*, while the ZmJAZ15 mutant showed greater susceptibility, highlighting the importance of the ZmCOI1a-ZmJAZ15 signaling module in maize immunity (Ma *et al.*, 2021).

**Resistance to abiotic stress:** Abiotic stress tolerance in maize has been significantly improved using CRISPR/Cas9 technology. Genes like ZmALS1 and ZmALS2, which are involved in branched-chain amino acid biosynthesis, have been edited to enhance herbicide resistance. Double mutations in these genes have resulted in up to 15-fold resistance to chlorsulfuron (Li *et al.*, 2020). Similarly, ZmHKT1, a gene linked to salt tolerance, has been targeted to enhance root-to-shoot Na<sup>+</sup> transport, improving salt tolerance in maize (Zhang *et al.*, 2020). Recent reviews (Mohamed *et al.*, 2024) highlight targeted genes such as ZmTMS5 for heat tolerance, ARGOS8 for drought resistance, and MS26 for herbicide resistance.

**Yield and quality improvement:** Yield enhancement in maize involves optimizing plant architecture and photosynthetic efficiency. The ZmACO2 gene, known for stimulating meristem and ear development, has been edited to increase kernel number, ear weight, and ear length, resulting in a 13.4% increase in grain yield per ear (Ning *et al.*, 2021). High-density planting strategies also benefit from CRISPR/Cas9 modifications that impact leaf angle, stem height, and overall plant structure, which are crucial for maximizing yield (Li *et al.*, 2017; Li *et al.*, 2020; Mansfield and Mumm, 2014). To improve nutritional value and quality, genes like ZmWAX1, ZmSH2, ZmZIP22, ZmBADH2a, and ZmBADH2b have been edited to enhance specific nutrition, flavor, and aroma of maize (Jiang *et al.*, 2022). In summary, CRISPR/Cas9 technology is revolutionizing maize crop improvement by addressing key challenges related to yield, stress resistance, and quality. This precision genome-editing tool enables the development of maize varieties that are more productive, resilient, and nutritionally superior, contributing to sustainable agricultural practices and global food security.

**CRISPR/Cas9 and Wheat Crop: Advancing wheat production through precision genome editing:** Wheat (*Triticum* spp.) is a crucial staple crop that feeds approximately 33% of the global population. With annual cultivation spanning over 217 million hectares, it is the most widely grown crop worldwide, providing about 20% of human calories. Enhancing wheat production, quality, and resilience is critical as food security becomes increasingly pressing due to global population growth and environmental

challenges. CRISPR/Cas9 technology has been extensively utilized to improve wheat traits, including grain production, quality, disease resistance, and tolerance to abiotic stresses such as drought, salinity, cold, osmotic pressure, and metal toxicity (Elsharawy and Refat, 2023). Food losses due to biotic, abiotic, and anthropogenic factors pose significant challenges. Increasing demand coupled with diminishing natural resources exacerbates the situation, with major issues including pests, diseases, climate change, and soil degradation. Minimizing food losses is essential for ensuring food security and supporting sustainable development as the global population is projected to reach 11 billion by 2080 (Junaid and Gokce, 2024).

**Resistance to biotic stress:** Biotic stress, caused by pathogens and pests, depletes plant nutrients, weakening plants and potentially leading to their death. This stress contributes to significant pre- and post-harvest agricultural losses (Shlibak *et al.*, 2021). Out of over 200 identified diseases and pests affecting wheat, about 50 are economically significant due to their impact on crop yield and farmers' income (Randhawa *et al.*, 2019). Major wheat diseases include powdery mildew, head blight, stem rust, leaf rust, and stripe rust (Kazerani *et al.*, 2022). CRISPR/Cas9 technology has proven invaluable in enhancing wheat resistance to these threats. For instance, the TaPDIL5-1 gene, involved in protein folding and disulfide bond formation, has been edited to confer resistance to wheat yellow mosaic virus (Kan *et al.*, 2022). The TaCIPK14 gene, part of the CBL-interacting protein kinase family, has been targeted to improve resistance to stripe rust, highlighting the role of the ZmCOI1a-ZmJAZ15 signaling module in maize immunity (Liu *et al.*, 2023; He *et al.*, 2023). Additionally, CRISPR/Cas9 has been used to knock down TaNFXL1, a gene involved in defense responses, to create wheat varieties with enhanced resistance to *Fusarium graminearum* (Brauer *et al.*, 2020).

**Resistance to abiotic stress:** Abiotic stresses such as heavy metals, salinity, drought, nutrient deficiencies, extreme temperatures, and pesticide contamination significantly impact agricultural output and global food security. These stresses affect plant photosynthetic efficiency, including chlorophyll production and gas exchange. CRISPR/Cas9 technology is employed to improve wheat's tolerance to such stresses. For example, the TaERF3 gene has been targeted to enhance both salt and drought tolerance (Debbarma *et al.*, 2019). The TaMBF1c gene, part of the multiprotein bridging factor 1 gene family, has been modified to improve wheat's response to cold, salinity, and drought (Tian *et al.*, 2022). Similarly, the Sal1 gene has been edited to boost drought tolerance (Abdallah *et al.*, 2022).

**Yield improvement:** CRISPR/Cas9 technology is also used to enhance wheat yield and quality by modifying various agronomic traits. Grain hardness, which affects milling energy and quality, is one such trait. The puroindoline b gene (Pinb) on chromosome 5DS influences wheat grain texture,



with CRISPR/Cas9 being used to improve this trait (Elsharawy and Refat, 2023). Furthermore, editing the waxy genes (WxA1, WxB1, and WxD1) has reduced amylose content, enhancing wheat flour's whiteness and opacity (Maryami *et al.*, 2020; Zhang *et al.*, 2021). For those affected by celiac disease, CRISPR/Cas9 has been employed to create wheat lines with inactivated gluten genes to reduce gluten, which could lower acrylamide content in wheat-based foods (Raffan *et al.*, 2021). Editing the TaIPK1 gene has led to reduced phytic acid and increased iron and zinc levels in wheat grains (Ibrahim *et al.*, 2022). The Tamyb10 gene, associated with pre-harvest sprouting resistance, has been restored using CRISPR/Cas9 to enhance wheat resilience (Zhu *et al.*, 2023). CRISPR/Cas9 has also improved several agronomic traits by modifying the miRNA156 recognition element in TaSPL13 and the TaARF15-A1 gene, which regulates senescence, leading to better grain ripening and foliage management (Gupta *et al.*, 2023; Li *et al.*, 2023). Recent studies have targeted the TaRPK1 gene to further boost wheat yield (Rahim *et al.*, 2024). Additional details on CRISPR/Cas9-targeted genes for wheat yield improvement are provided in Table 3. In summary, CRISPR/Cas9 technology is transforming wheat breeding by addressing key challenges related to biotic and abiotic stress resistance, yield, and grain quality, ultimately contributing to enhanced food security and agricultural sustainability.

**Conclusion:** CRISPR/Cas9 is revolutionizing crop science by providing precise, efficient, and cost-effective tools for editing plant DNA. This technology enables scientists to make targeted modifications to the genetic makeup of crops, enhancing their yield, nutritional value, and resistance to diseases and environmental stresses. Its significant impacts include improved crop traits, better pest and disease resistance, increased tolerance to environmental stress, accelerated breeding programs, and reduced reliance on traditional GMOs. By directly introducing desirable traits, CRISPR/Cas9 streamlines breeding processes, bypassing lengthy cross-breeding cycles. As a result, this technology plays a crucial role in promoting sustainable agriculture and environmental sustainability.

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## REFERENCES

- Abd El Mageed, T.A., W. Semida, K.A. Hemida, M.A.H. Gyushi, M.M. Rady, A. Abdelkhalik, O. Merah, M. Brestic, H.I. Mohamed, A.E. Sabagh and M.T. Abdelhamid. 2023. Glutathione-mediated changes in productivity, photosynthetic efficiency, osmolytes, and antioxidant capacity of common beans (*Phaseolus vulgaris*) grown under water deficit. *Peer J.* 11:e15343.
- Abdallah, N.A., H. Elsharawy, H.A. Abulela, R. Thilmoney, A.A. Abdelhadi and N.I. Elarabi. 2022. Multiplex CRISPR/Cas9-mediated genome editing to address drought tolerance in wheat. *GM Crop and Food* 6:1-17.
- Alam, M.S., J. Kong, R. Tao, T. Ahmed, M.d. Alamin, S.S. Alotaibi, N.R. Abdelsalam and J.H. Xu. 2022. CRISPR/Cas9 mediated knockout of the OsbHLH024 transcription factor improves salt stress resistance in rice (*Oryza sativa* L.). *Plants* 11:e1184.
- Ammar, A., M.N. Khalid and I. Amjad. 2023. maize crop transformation with CRISPR: State-of-the-art and future directions. *Trend in Biotechnology and Plant Sciences* 1:16-28.
- Ariizumi, T., Y. Shinozaki and H. Ezura. 2013. Genes that influence yield in tomato. *Breeding Science* 63:3-13.
- Bhaya, D., M. Davison and R. Barrangou. 2011. CRISPR-Cas systems in bacteria and archaea: Versatile small RNAs for adaptive defense and regulation. *Annual Reviews of Genetics* 45:273-297.
- Bigliardi, B. and F. Galati. 2013. Innovation trends in the food industry: The case of functional foods. *Trend in Food Science and Technology* 31:118-129.
- Brauer, E.K., M. Balcerzak, H. Rocheleau, W. Leung, J. Scherthaner, R. Subramaniam and T. Ouellet. 2020. Genome editing of a deoxynivalenol-induced transcription factor confers resistance to *Fusarium graminearum* in wheat. *Molecular Plant-Microbe Interactions* 33:553-560.
- Chen, L., D. Yang, Y. Zhang, L. Wu, Y. Zhang, L. Ye, C. Pan, Y. He, L. Huang, Y-L Ruan and G. Lu. 2018. Evidence for a specific and critical role of mitogen-activated protein kinase 20 in uni-to-binucleate transition of microgametogenesis in tomato. *New Phytologist* 219:176-194.
- Debbarma, J., Y.N. Sarki, B. Saikia, H.P.D. Boruah, D.L. Singha and C. Chikkaputtaiah. 2019. Ethylene response factor (ERF) family proteins in abiotic stresses and CRISPR-Cas9 genome editing of ERFs for multiple abiotic stress tolerance in crop plants: A review. *Molecular Biotechnology* 61:153-172.
- Elsharawy, H. and M. Refat. 2023. CRISPR/Cas9 genome editing in wheat: Enhancing quality and productivity for global food security—a review. *Functional & Integrative Genomics* 23: 265.



- Gao, C. 2018. The future of CRISPR technologies in agriculture. *Nature Reviews Molecular Cell Biology* 19:275-276.
- Gupta, A., L. Hua, Z. Zhang, B. Yang and W. Li. 2023. CRISPR-induced miRNA156-recognition element mutations in TaSPL13 improve multiple agronomic traits in wheat. *Plant Biotechnology Journal* 21:536-548.
- Gurunathan, S., B.R. Ramadoss, A. Singaram and D. Arulbalachandran. 2023. CRISPR/Cas9 genome editing tool for rice crop improvement. *Cornus Biology* 1:1-6.
- He, F., C. Wang, H. Sun, S. Tian, G. Zhao, C. Liu, C. Wan, J. Guo, X. Huang, G. Zhan, X. Yu, Z. Kang and J. Guo. 2023. Simultaneous editing of three homoeologues of TaCIPK14 confers broad-spectrum resistance to stripe rust in wheat. *Plant Biotechnology Journal* 21:354-368.
- Hovarth, P. and R. Barangu. 2010. CRISPR/Cas, the immune system of bacteria and archaea. *Science* 327:167-170.
- Hu, Z., J. Li, S. Ding, F. Cheng, X. Li, Y. Jiang, J. Yu, C.H. Foyer and K. Shi. 2021. The protein kinase CPK28 phosphorylates ascorbate peroxidase and enhances thermotolerance in tomato. *Plant Physiology* 186:1302-1317.
- Ibrahim, S., B. Saleem, N. Rehman, S.A. Zafar, M.K. Naeem and M.R. Khan. 2022. CRISPR/Cas9 mediated disruption of Inositol Pentakisphosphate 2-Kinase 1 (TaIPK1) reduces phytic acid and improves iron and zinc accumulation in wheat grains. *Journal of Advanced Research* 37:33-41.
- Jain, P., P.K. Singh, R. Kpoor, A. Khanna, A.U. Solanke, S.G. Krishnan, A.K. Singh, V. Sharma and T.R. Sharma. 2017. Understanding host-pathogen interactions with expression profiling of NILs carrying rice-blast resistance Pi9 gene. *Frontier in Plant Science* 8:93.
- Jansen, R., J.D.A.V. Embden, W. Gastra and L.M. Schouls. 2002. Identification of genes that are associated with DNA repeats in prokaryotes. *Molecular Microbiology* 43:1565-1575.
- Jiang, Y., K. Sun and X. An. 2022. CRISPR/Cas system: Applications and prospects for maize. *ACS Agricultural Science & Technology* 2:174-183.
- Jinek, M., K. Chylinski, I. Fonfara, M. Hauer, J.A. Doudna and E. Charpentier. 2012. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* 337:816-821.
- Junaid, M.D., and A.F. Gokce. 2024. Global agricultural losses and their cause. *Bulletin of Biological and Allied Sciences Research* 9:66.
- Kan, J., Y. Cai, C. Cheng, C. Jiang, Y. Jin and P. Yang. 2022. Simultaneous editing of host factor gene TaPDIL5-1 homoeoalleles confers wheat yellow mosaic virus resistance in hexaploid wheat. *New Phytologist* 234:340-344.
- Kazerani, H.S.B., H. A. Falahi, M.A. Dehghan, S.M. Alegh, A.R. Dadras, M. Katouzi and A. Mastinu. 2022. Association analysis of yellow rust, fusarium head blight, tan spot, powdery mildew, and brown rust horizontal resistance genes in wheat. *Physiological and Molecular Plant Pathology* 118:101808.
- Kim, M.S., V.T. Le, Y.J. Jung, K.k. Kang and Y.G. Cho. 2024. OsPUB9 gene edited by CRISPR/Cas9 enhanced resistance to bacterial leaf blight in rice (*Oryza sativa* L.). *International Journal of Molecular Biology* 25:7145.
- Kim, M-S., S-R. Ko, Y.J. Jung, K-K. Kang, Y.J. Lee and Y-G. Cho. 2023. Knockout mutants of OsPUB7 generated using CRISPR/Cas9 revealed abiotic stress tolerance in rice. *International Journal of Molecular Sciences* 24:5338.
- Kim, Y.A., H. Moon and C.J. Park. 2019. CRISPR/Cas9-targeted mutagenesis of Os8N3 in rice to confer resistance to *Xanthomonas oryzae* pv. *oryzae*. *Rice* 12:67.
- Kuang, Y., S. Li, B. Ren, F. Yan, C. Spetz, X. Li, X. Zhou and H. Zhou. 2020. Base-editing-mediated artificial evolution of OsALS1 in planta to develop novel herbicide-tolerant rice germplasms. *Molecular Plant* 13:565-572.
- Kumar, V.V.S., R.K. Verma, S.K. Yadav, P. Yadav, A. Watts, M.V. Rao and V. Chinnusamy. 2020. CRISPR-Cas9 mediated genome editing of drought and salt tolerance (OsDST) gene in indica mega rice cultivar MTU1010. *Physiology and Molecular Biology of Plants* 26: 1099-1110.
- Li, C., C. Liu, X. Qi, Y. Wu, X. Fei, L. Mao, B. Cheng, X. Li and C. Xie. 2017. RNA-guided Cas9 as an in vivo desired-target mutator in maize. *Plant Biotechnology Journal* 15:1566-1576.
- Li, H., H. Liu, C. Hao, T. Li, Y. Liu, X. Wang, Y. Yang, J. Zheng and X. Zhang. 2023. The auxin response factor TaARF15-A1 negatively regulates senescence in common wheat (*Triticum aestivum* L.). *Plant Physiology* 191:1254-1271.
- Li, Q., G. Wu, Y. Zhao, B. Wang, B. Zhao, D. Kong, H. Wei, C. Chen and H. Wang. 2020. CRISPR/Cas9-mediated knockout and overexpression studies reveal a role of maize phytochrome C in regulating flowering time and plant height. *Plant Biotechnology Journal* 15: 2520-2532.
- Li, R., L. Zhang, L. Wang, L. Chen, R. Zhao, J. Sheng and L. Shen. 2018. Reduction of tomato-plant chilling tolerance by CRISPR-Cas9-mediated SICBF1 mutagenesis. *Journal of Agricultural and Food Chemistry* 66:9042-9051.
- Liu, L., J. Zhang, J. Xu, Y. Li, L. Guo, Z. Wang, X. Zhang, B. Zhao, Y.D. Guo and N. Zhang. 2020. CRISPR/Cas9 targeted mutagenesis of SILBD40, a lateral organ boundaries domain transcription factor, enhances drought tolerance in tomato. *Plant Biology* 301:110683.





- Liu, Q., F. Yang, J. Zhang, H. Liu, S. Rahman, S. Islam, W. Ma and M. She. 2021. Application of CRISPR/Cas9 in crop quality improvement. *International Journal of Crop Science* 22:4206.
- Liu, X., X. Wang, C. Yang, G. Wang, B. Fan, Y. Shang, C. Dang, C. Xie and Z. Wang. 2023. Genome-wide identification of TaCIPK gene family members in wheat and their roles in host response to *Blumeria graminis* F. sp. tritici infection. *International Journal of Biological Macromolecules* 248:125691.
- Liu B., Z. Guo, K. Zhong, T. Osire, Y. Sun and L. Jiang. 2023. State of the art in CRISPR/Cas system-based signal conversion and amplification applied in the field of food analysis. *Trends in Food Science & Technology* 135:174-189.
- Ly, L.K., T.M. Ho, T.P. Bui, L.T. Nguyen, Q. Phan, N.T. Le, L.T.M. Khuat, L.H. Le, H.H. Chu, N.B. Pham and P.T. Do. 2024. CRISPR/Cas9 targeted mutations of OsDSG1 gene enhanced salt tolerance in rice. *Functional & Integrative Genomics* 24:70.
- Ma, L., Y. Sun, X. Ruan, P-C. Huang, S. Wang, S. Li, Y. Zhou, F. Wang, Y. Cao, Q. Wang, Z. Wang, M.V. Kolomiets and X. Gao. 2021. Genome-wide characterization of jasmonates signaling components reveals the essential role of ZmCOI1a-ZmJAZ15 action module in regulating maize immunity to gibberella stalk rot. *International Journal of Molecular Sciences* 22:870.
- Ma, X. and Y.G. Liu. 2016. CRISPR/Cas9-based multiplex genome editing in monocot and dicot plants. *Current Protocol in Molecular Biology* 115: 31.6.1-31.6.21.
- Mansfield, B.D. and R. H. Mumm. 2014. Survey of plant density tolerance in U.S. maize germplasm. *Crop Science* 54: 157-173.
- Mao, Y., J.R. Botella, Y. Liu and J.K. Zhu. 2019. Gene editing in plants: progress and challenges. *National Science Review* 6:421-437.
- Maryami, Z., M.R. Azimi, C. Guzman, S. Dreisigacker and G. Najafian. 2020. Puroindoline (Pina-D1 and Pinb-D1) and waxy (Wx-1) genes in Iranian bread wheat (*Triticum aestivum* L.) landraces. *Biotechnology and Biotechnological Equipment* 34:1019-1027.
- Miller, A.J. and B.L. Gross. 2011. From forest to field: perennial fruit crop domestication. *American Journal of Botany* 98:1389-1414.
- Mohamed, H.I., A. Khan and A. Basit. 2024. CRISPR-Cas9 system mediated genome editing technology: An ultimate tool to enhance abiotic stress in crop plants. *Journal of Soil Science and Plant Nutrition* 24:1799-1822.
- Ning, Q., Y. Jian, Y. Du, Y. Li, X. Shen, H. Jia, R. Zhao, J. Zhan, F. Yang, D. Jackson, L. Liu and Z. Zhang. 2021. An ethylene biosynthesis enzyme controls quantitative variation in maize ear length and kernel yield. *Nature Communication* 12:5832.
- Parmar, N., K.H. Singh, D. Sharma, L. Singh, P. Kumar, J. Nanjundan, Y.J. Khan, D.K. Chauhan and A.K. Thakur. 2017. Genetic engineering strategies for biotic and abiotic stress tolerance and quality enhancement in horticultural crops: a comprehensive review. *3Biotech* 7:239.
- Pathi, K.M., P. Rink, N. Budhagatapalli, R. Betz, I. Saado, S. Hiekel, M. Becker, A. Djamei and J. Kumlehn. 2020. Engineering smut resistance in maize by site-directed mutagenesis of LIPOXYGENASE 3. *Frontier in Plant Sciences* 11:543895.
- Perk, E.A., A.A.D. Palma, S. Colman, O. Mariani, I. Cerrudo, J.M. D'Ambrosio, L. Robuschi, M.A. Pombo, H.G. Rosli, F. Villareal and A. M. Laxalt. 2023. CRISPR/Cas9-mediated phospholipase C 2 knock-out tomato plants are more resistant to *Botrytis cinerea*. *Planta* 257:117.
- Podevin, N., H.V. Davies, F. Hartung, F. Nogue and J.M. Casacuberta. 2013. Site-directed mutagenesis: a paradigm shifts in predictable, knowledge-based plant breeding. *Trends in Biotechnology* 31:375-383.
- Raffan, S., C. Sparks, A. Huttly, L. Hyde, D. Martignago, A. Mead, S.J. Hanley, P.A. Wilkinson, G. Barker, K. J. Edwards, T.Y. Curtis, S. Usher, O. Kosik and N.G. Halford. 2021. Wheat with greatly reduced accumulation of free asparagine in the grain, produced by CRISPR/Cas9 editing of asparagine synthetase gene TaASN2. *Plant Biotechnology Journal* 19:1602-1613.
- Rahim, A.A., M. Uzair, N. Rehman, S. Fiaz, K.A. Attia, A.M. Abushady, S.H. Yang and M.R. Khan. 2024. CRISPR/Cas9 mediated TaRPK1 root architecture gene mutagenesis confers enhanced wheat yield. *Journal of King Saud University – Science* 36:103063.
- Randhawa, M.S., S. Bhavani, P.K. Singh, J. Huerta-Espino and R.P. Singh. 2019. Disease Resistance in Wheat: Present Status and Future Prospects. In: Wani, S.H. (eds) *Disease Resistance in Crop Plants*. Springer, Cham. [https://doi.org/10.1007/978-3-030-20728-1\\_4](https://doi.org/10.1007/978-3-030-20728-1_4)
- Rasheed, A., R.A. Gill, M.U. Hassan, A. Mehmood, S. Qari, Q.U. Zaman, M. Ilyas, M. Aamer, M. Batool, H. Li and Z. Wu. 2021. A Critical Review: Recent advancements in the use of CRISPR/cas9 technology to enhance crops and alleviate global food crises. *Current Issues in Molecular Biology* 43:1950-1976.
- Reiger, M. 2006. *Introduction to fruit crops* (1<sup>st</sup> Ed.). CRC Press, Boca Raton. <https://doi.org/10.1201/9781482298055>
- Ricroch, A., P. Clairand and W. Harwood. 2017. Use of CRISPR systems in plant genome editing: toward new opportunities in agriculture. *Emerging Topics in Life Sciences* 1:169-182.
- Romero, F.M. and A. Gatica-Arias. 2019. CRISPR/Cas9: Development and application in rice breeding. *Rice Science* 26:265-281.



- Satpute, M.R. and P.S.M. Jagdale. 2016. Color, size, volume, shape and texture feature extraction. *International Research Journal of Engineering and Technology* 3:703-708.
- Sethi, S. 2024. CRISPR/Cas-Mediated Multiplex Gene Editing in Tomato (*Solanum Lycopersicum* L.). In: Kumar, A., Arora, S., Ogita, S., Yau, YY., Mukherjee, K. (eds) *Gene Editing in Plants*. Springer, Singapore. [https://doi.org/10.1007/978-981-99-8529-6\\_28](https://doi.org/10.1007/978-981-99-8529-6_28)
- Shan, Q., Y. Wang, J. Li and C. Gao. 2014. Genome editing in rice and wheat using the CRISPR/Cas system. *Nature Protocol* 9:2395-2410.
- Shlibak, A.A., M. Orgec and N. Zencirci. 2021. Wheat landraces versus resistance to biotic and abiotic stresses, pp. 193–214. In: Zencirci, N., F.S. Baloch, E. Habyarimana, G. Chung (Ed.), *Wheat Landraces*. Springer, Cham.
- Shu, P., Y. Li, L. Xiang, J. Sheng and L. Shen. 2023. SINPR1 modulates chilling stress resistance in tomato plant by alleviating oxidative damage and affecting the synthesis of ferulic acid. *Scientia Horticulturae* 307:111486.
- Song, G., M. Jia, K. Chen, X. Kong, B. Khattak, C. Xie, A. Li and L. Mao. 2016. CRISPR/Cas9: A powerful tool for crop genome editing. *The Crop Journal* 4:75-82.
- Song, J., L. Tang, H. Fan, X. Xu, X. Peng, Y. Cui and J. Wang. 2024. Enhancing yield and improving grain quality in Japonica rice: Targeted EHD1 Editing via CRISPR-Cas9 in low-latitude adaptation. *Current Issues in Molecular Biology* 46:3741-3751.
- Tashkandi, M., Z. Ali, F. Aljedaani, A. Shami and M.M. Mahfouz. 2018. Engineering resistance against tomato yellow leaf curl virus via the CRISPR/Cas9 system in tomato. *Plant Signaling & Behavior* 13.
- Tavora, F.T.P.K., A.C. Meunier, A. Vernat, M. Portefaix, J. Milazzo, H. Adreit, D. Thareau, O.L. Franco and A. Mehta. 2022. CRISPR/Cas9-targeted knockout of rice susceptibility genes OsDJA2 and OsERF104 reveals alternative sources of resistance to *Pyricularia oryzae*. *Rice Science* 29:535-544.
- Thomazella, D.P.D.T., Q. Brail, D. Dahlbeck, B. Staskawicz. 2016. CRISPR-Cas9 mediated mutagenesis of a DMR6 ortholog in tomato confers broad-spectrum disease resistance. *bioRxiv* 064824; doi: <https://doi.org/10.1101/064824>.
- Tian, X., Z. Qin, Y. Zhao, J. Wen, T. Lan, L. Zhang, F. Wang, D. Qin, K. Yu, A. Zhao, Z. Hu, Y. Yao, Z. Ni, Q. Sun, I.D. Smet, H. Peng and M. Xin. 2022. Stress granule-associated TaMBF1c confers thermotolerance through regulating specific mRNA translation in wheat (*Triticum aestivum*). *New Phytologist* 233:1719-1731.
- Wang, T., Z. Deng, X. Zhang, H. Wang, Y. Wang, X. Liu, S. Liu, F. Xu, T. Li, D. Fu, B. Zhu, Y. Luo and H. Zhu. 2018. Tomato DCL2b is required for the biosynthesis of 22-nt small RNAs, the resulting secondary siRNAs, and the host defense against ToMV. *Horticulture Research* 5:62. <https://doi.org/10.1038/s41438-018-0073-7>
- Wang, W., W. Wang, Y. Pan, C. Tan, H. Li, Y. Chen, X. Liu, J. Wei, N. Xu, Y. Han, H. Gu, R. Ye, Q. Ding and C. Ma . 2022. A new gain-of-function OsGS2/GRF4 allele generated by CRISPR/Cas9 genome editing increases rice grain size and yield. *The Crop Journal* 10:1207-1212.
- Wu, Q., M. Regan, H. Furukawa and D. Jackson. 2018. Role of heterotrimeric Gα proteins in maize development and enhancement of agronomic traits. *PLOS Genetics* 14:e1007374.
- Xing, Y. and Q. Zhang. 2010. Genetic and molecular bases of rice yield. *Annual Review of Plant Biology* 61:421-442.
- Yang, I., Y. Zhang, Z. Sun, Z. Shen, Y. Li, Y. Guo, Y. Feng, S. Sun, M. Guo, Z. Hu and C. Yan. 2023. Knocking out OsAAP11 to improve rice grain quality using CRISPR/Cas9 system. *International Journal of Molecular Sciences* 24:14360.
- Yang, T.X., L. Deng, W. Zhao, R. Zhang, H.L. Jiang, Z-B. Ye, C-B. Li, C.Y. Li. 2019. Rapid breeding of pink-fruited tomato hybrids using the CRISPR/Cas9 system. *Journal of Genetics and Genomics* 46: 505-508.
- Yu, Y., M. Yu, S. Zhang, T. Song, M. Zhang, H. Zhou, Y. Wang , J. Xiang and X. Zhang. 2022. Transcriptomic identification of wheat AP2/ERF transcription factors and functional characterization of TaERF-6-3A in response to drought and salinity stresses. *International Journal of Molecular Science* 23:3272.
- Zafar, K., M.Z. Khan, I. Amin, Z. Mukhtar, S. Yasmin, M. Arif, K. Ejaz and S. Mansoor. 2020. Precise CRISPR-Cas9 mediated genome editing in super basmati rice for resistance against bacterial blight by targeting the major susceptibility gene. *Frontier in Plant Sciences* 11:575.
- Zetsche, B., J.S Gootenberg, O.O. Abudayyeh, A. Regev, E.V Koonin and F. Zhang. 2015. Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. *Cell* 163:759-771.
- Zhang, Z., X. Zhang, Z. Lin, J. Wang, H. Liu, L. Zhou, S. Zhong, Y. Li, C. Zhu, J. Lai, X. Li, J. Yu and Z. Lin. 2020. A large transposon insertion in the stiff1 promoter increases stalk strength in maize. *The Plant Cell* 32:152-165.
- Zhang, D., Z. Li and J-F. Li. 2016. Targeted gene manipulation in plants using the CRISPR/Cas technology. *Journal of Genetics and Genomics* 43: 251-262.
- Zhang, J., S. Liu, X. Zhu, Y. Chang, C. Wang, N. Ma, U. Wang, X. Zhang, J. Lyu and J. Xie. 2023. A comprehensive evaluation of tomato fruit quality and identification of volatile compounds. *Plant* 12:2947.
- Zhang, S., L. Wang, R. Zhao, W. Yu, R. Li, Y. Li, J. Sheng and L. Shen. 2018. Knockout of SIMAPK3 reduced disease resistance to *Botrytis cinerea* in tomato plants.



- Journal of Agricultural and Food Chemistry 66:8949-8956.
- Zhang, S., R. Zhang, J. Gao, G. Song, J. Li, W. Li, Y. Qi, Y. Li and G. Li. 2021. CRISPR/Cas9-mediated genome editing for wheat grain quality improvement. *Plant Biotechnology Journal* 19:1684-1686.
- Zhou, Y., S. Xu, N. Jiang, X. Zhao, Z. Bai, J. Liu, W. Yao, Q. Tang, G. Xiao, C. Lv, K. Wang, X. Hu, J. Tan and Y. Yang. 2022. Engineering of rice varieties with enhanced resistances to both blast and bacterial blight diseases via CRISPR/Cas9. *Plant Biotechnology Journal* 20: 876-885.
- Zhu, Y., Y. Li, Y. Fan, Y. Wang, P. Li, J. Xiong, Y. He, S. Cheng, X. Ye, F. Wang, J. Goodrich, J.K. Zhu, K. Wang, and C.J. Zhang. 2023. CRISPR/Cas9-mediated restoration of Tamyb10 to create pre-harvest sprouting-resistant red wheat. *Plant Biotechnology Journal* 21:665-667.

